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# THE RELATIVE EFFECTIVENESS OF FISSION NEUTRONS FOR GASTROINTESTINAL DAMAGE IN MICE: JEJUNAL CRYPT STEM CELL SURVIVAL

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TABLE OF CONTENTS



LIST OF FIGURES



TABLE

## FOREWORD
(Nontechnical summary)

A recent study has provided a method for quantitative determination of intestinal crypt stem cell survival following irradiation. This method was applied in the present study to assess the degree of jejunal crypt stem cell damage in mice resulting from acute exposure to fission neutrons or gamma rays at various dose rates. The survival data were analyzed using the multitarget single-hit cell survival theory. This theory provided survival curves which fit the data well. At low levels of cell survival, gamma rays were found to be approximately 20 percent more effective in destroying cells when delivered in a brief pulse of radiation than when delivered at lower dose rates (40 rads/minute or 250 rads/minute). This dose rate effect is due to the repair capability of the jejunal crypt stem cells. Low linear energy transfer (LET) radiations, such as gamma rays, cause various levels of sublethal cellular damage. At low gamma ray dose rates, much of this sublethal damage can be repaired by the cells before further damage occurs. Hence, crypt stem cell survival was found to be greater at low dose rates. However, little of the cellular damage produced by the high LET fission neutrons is reparable. Consequently, increased crypt stem cell survival was not observed at low neutron dose rates.

ABSTRACT

Mice were unilaterally irradiated in either a fission neutron or gamma ray field at various dose rates. Jejunal crypt stem cell survival was analyzed by the multi-target single-hit survival model, which yielded excellent fitting survival curves. At the 1 percent survival level, gamma rays were found to be 20 percent more effective in destroying cells when delivered in a brief pulse of radiation than when delivered at lower dose rates (40 rads/minute or 250 rads/minute). Increased cell survival was not observed for low dose rate neutrons. $D_o$ values obtained were consistent with the findings of similar studies; however, survival curve intercepts were found to be lower than previous estimates.

# I. INTRODUCTION

The response of the jejunal crypts of mice to pulsed doses of reactor-produced neutrons and gamma rays was described in a previous report.[12] Survival of a jejunal crypt following exposure to ionizing radiation is dependent upon the sensitivity of the proliferative cells of that crypt. The kinetics of this cellular renewal system have been reported in detail.[6] Each crypt contains a proliferative compartment comprising approximately 60 percent of the total crypt cell population. All cells in this compartment undergo mitosis and, as division occurs, the cells are forced up the sides of the crypt. Cell division ceases as the cells move upward into the maturation zone and onto the villus. The functional epithelial cells progress upward on the villus and are eventually extruded from its tip into the intestinal lumen.

Intestinal irradiation has been shown to interrupt this orderly renewal process by delaying or destroying proliferative cells in the process of division.[6] These cells have a remarkable capacity for repair, however, and nearly all of the proliferative cells must be destroyed to effect irreparable damage to the crypt.[4] This repair capability is further evidenced by the fact that fractionated or low dose rate radiations cause less cellular damage than do single or high dose rate radiations.[5,9]

The purpose of this study was to investigate the sensitivity of the jejunal crypt stem cells of mice to reactor-produced neutrons and gamma rays at various dose rates. The microcolony assay technique of Withers and Elkind[10] was used to determine the numbers of surviving crypt stem cells. These data were analyzed using the multitarget single-hit cell survival theory.[3]

## II. MATERIALS AND METHODS

Male $C_3D_2F_1$ mice,* 4-5 months of age, were exposed in groups of 10 to 20 to either a fission neutron or gamma ray enriched radiation field, as previously described.[12] Exposures were conducted at 40 rads/minute, 250 rads/minute, and in the pulse mode (approximately $10^7$ rads/minute).

The percentages of jejunal crypts destroyed by radiation were determined as previously described,[12] and the numbers of jejunal crypt stem cells surviving a given dose of radiation were then calculated, as follows.[10] If it is assumed that the cells survive independently of one another and that one surviving stem cell in a crypt is sufficient to regenerate a viable crypt, then the number of surviving stem cells can be calculated from Poisson statistics. For an average of y surviving stem cells per crypt, the proportion of crypts having no surviving cells is $e^{-y}$. This proportion is (142 - x)/142, where x is the average number of viable crypts per jejunal circumference and 142 is the average number of crypts per jejunal circumference of the nonirradiated mice.[12] Hence, the average number of surviving stem cells per cross section (N) is

$$N = -142 \ln \frac{142 - x}{142} . \quad (1)$$

The multitarget single-hit cell survival model[3] predicts that the number of cells N surviving a dose of radiation D is

$$N = N_o \left[1 - (1 - e^{-D/D_o})^n\right] \quad (2)$$

where $N_o$ is the initial number of cells, n is the extrapolation number, or the number

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of targets in the cell that must be "hit" to cause cell death, and $D_0$, a characteristic of the radiation sensitivity of the cell, is the dose which reduces cell survival by a factor of $e^{-1}$ along the exponential portion of the survival curve. For small surviving fractions of cells, equation (2) reduces to

$$N = n\, N_0\, e^{-D/D_0} \quad . \tag{3}$$

The logarithmic form of equation (3) is linear and is generally applied in analysis of cell survival data. The data were not consistent with the reduced form of equation (2) since it appeared that some of the data belonged to the "shoulder" or nonlinear portion of the survival curve. Hence, equation (2) was applied directly and fitted by computer using a nonlinear least squares approach,[1] with $N_0$, n, and $D_0$ as fitting parameters.

## III. RESULTS

The numbers of surviving crypt stem cells per jejunal circumference, calculated as described above, are shown in Figures 1 - 3. Other reports[8] have estimated the intercept value of the survival curves to be 20,000 cells per circumference. The maximum level of postirradiation cell survival which occurred was approximately 400 cells per jejunal circumference. This survival level is 2 percent of the estimated intercept value and should therefore lie in the linear portion of the curve represented by equation (3). However, tests of linearity were performed and the data were found to be inconsistent with this hypothesis. When equation (2) was fitted directly to the data, the intercept values obtained ranged from approximately 300 to 900. The choice of a common midrange intercept value of 400 stem cells per jejunal circumference resulted in the excellent fitting curves shown in Figures 1 - 3. The $D_0$ and n values obtained, using this common intercept, are listed in Table I.

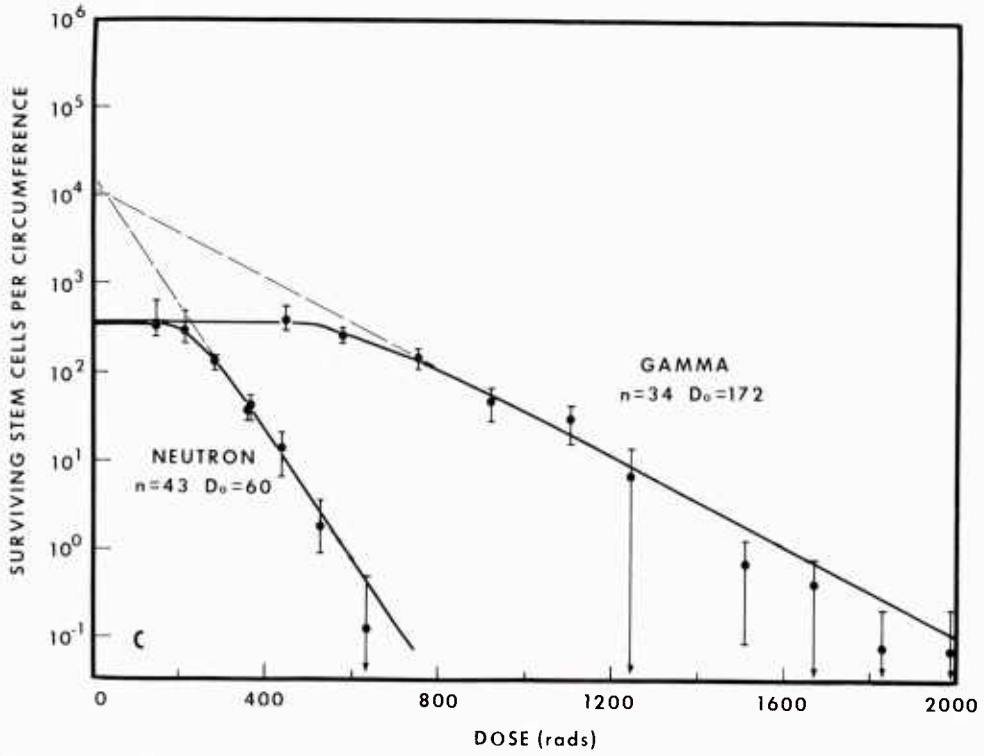


Figure 1. Jejunal crypt stem cell survival after 40 rads/minute neutron and gamma irradiation. Bars indicate one standard deviation ranges.

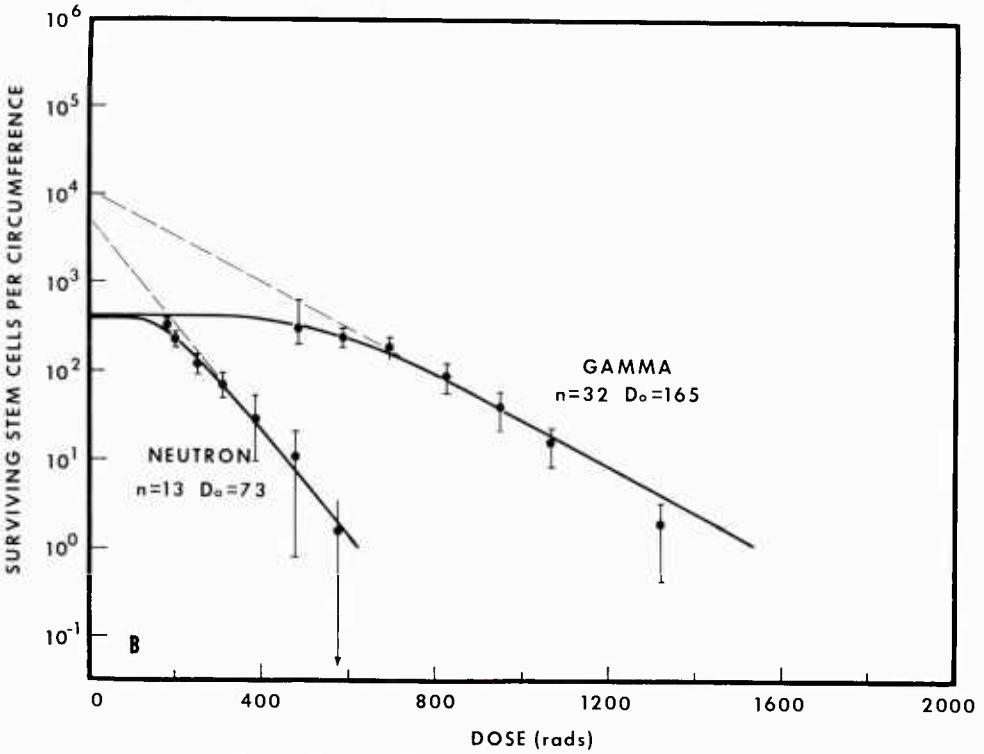


Figure 2. Jejunal crypt stem cell survival after 250 rads/minute neutron and gamma irradiation. Bars indicate one standard deviation ranges.

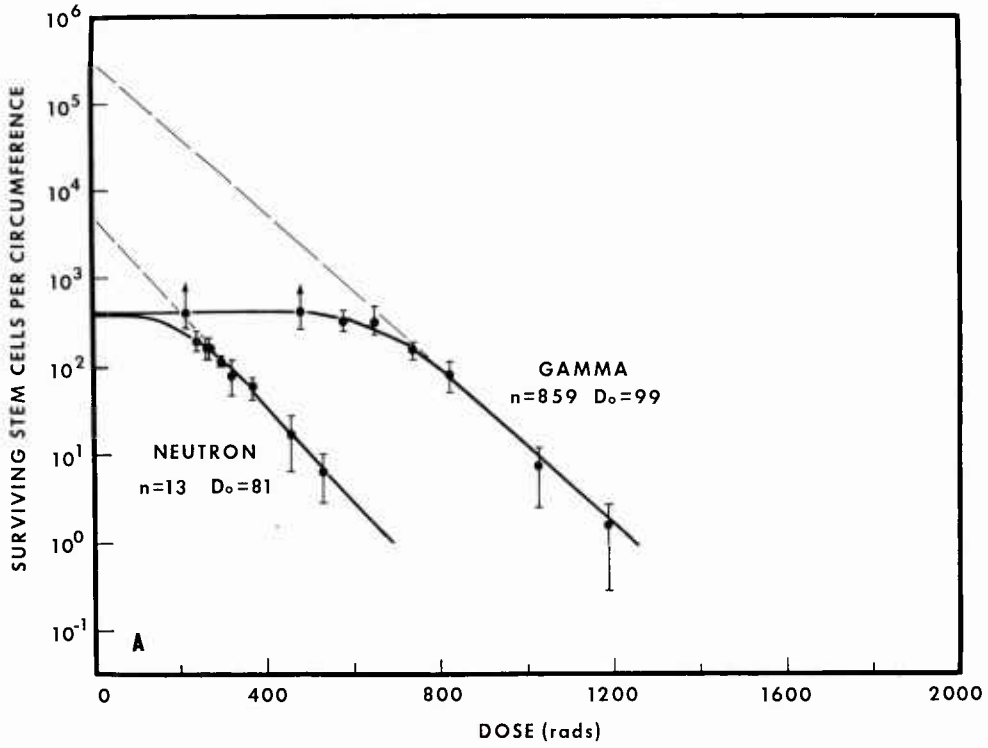


Figure 3. Jejunal crypt stem cell survival after pulsed neutron and gamma irradiation. Bars indicate one standard deviation ranges.

Table I. Extrapolation Numbers (n) and $D_o$ Values for Jejunal Crypt Stem Cell Survival Curves

| | n | $D_o$ (rads) |
|---|---|---|
| Gamma (40 rads/minute) | 34.3 ± 9.7* | 172.5 ± 10.3 |
| Gamma (250 rads/minute) | 31.7 ± 6.7 | 165.3 ± 7.7 |
| Gamma (pulsed) | 859 ± 362 | 98.8 ± 5.5 |
| Neutron (40 rads/minute) | 43.3 ± 12.2 | 60.4 ± 3.3 |
| Neutron (250 rads/minute) | 13.0 ± 2.7 | 72.8 ± 4.2 |
| Neutron (pulsed) | 12.8 ± 3.1 | 80.9 ± 5.3 |

* Standard deviation

No dose rate dependence was observed for fission neutrons or gamma rays at levels of cell survival greater than 20 percent. However, at low levels of stem cell survival (i.e., 1 percent) the pulsed gamma rays were approximately 20 percent more effective than the low dose rate gamma rays, while a slight reverse dose rate effect was observed for neutrons.

## IV. DISCUSSION

The multitarget single-hit cell survival theory has provided excellent fitting jejunal crypt stem cell survival curves, especially in the low dose or "shoulder" portion of the curves. However, it was observed at high doses that most of the data points fell below the predicted survival line. This suggests that possibly a continuously bending survival curve would better describe the data. This phenomenon has also been observed by others,[7] but was not further investigated in the present study.

Withers et al.[8] have reported the $D_0$ value of 14 MeV neutrons delivered at 15 - 20 rads/minute to be 100 rads. Our values of 60.4, 72.8, and 80.9 rads for the 40 rads/minute, 250 rads/minute, and pulsed fission neutrons, respectively, are considerably lower than this value. This may be explained by the fact that the fission neutron spectrum contains fewer low linear energy transfer (LET) components than do the 14 MeV neutrons. Withers et al.[11] have also reported the $D_0$ of californium-252 neutrons as 72 rads. Since the energy spectra of californium neutrons and reactor-produced fission neutrons are similar, this value agrees well with our results. Our values of 172 and 165 rads for the 40 rads/minute and 250 rads/minute gamma rays agree well with previously reported low LET $D_0$ values.[5, 8, 11] However, the $D_0$ of 98.8 rads for the pulsed gamma rays is lower than any previously reported, and is

probably due to the extremely high dose rate employed plus the slight neutron component of the gamma ray field.[12]

The survival curve intercept values found in this study differ markedly from other predictions. The number of stem cells per jejunal crypt of mice has been estimated to be 130 to 160.[4,8] The low intercept values found in this study indicate that there are only about three stem cells per crypt. This small number of crypt stem cells is indeed feasible. The generation cycle time for proliferative crypt cells is 10 - 13 hours.[6] Since crypt cell repopulation after irradiation occurs within 2 - 3 days,[2] it is possible that as many as five to seven mitotic cycles can occur before the original progeny of stem cell division leave the crypt proliferative zone. If there were but three initial crypt stem cells whose dividing and differentiating progeny underwent an additional six mitotic divisions before leaving the crypt proliferative zone, then the total proliferative crypt cell population would be 192, in reasonable agreement with the above estimates. Also, the net cell production rate would be approximately 15 cells per hour, agreeing well with the reported production rate of 10 cells per crypt per hour.[6] Hence, the survival curve intercept values found in this study, although much lower than previous estimates, are not in disagreement with the known kinetic properties of the crypt cell renewal system of the mouse.

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